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NUCLEIC ACID SEQUENCES CHARACTERISTIC OF HEMATOPOIETIC STEM CELLS

BACKGROUND OF THE INVENTION

Hematopoietic Cells

The processes involved in the formation and differentiation of blood cells are collectively called hematopoiesis. There are various types of blood cells, such as red blood cells (erythrocytes), white blood cells (e.g., neutrophils, basophils, and eosinophils and monocytes), lymphocytes, and platelets. Red blood cells are involved in respiratory gas (oxygen and carbon dioxide) transport. White blood cells function to protect the body from infectious diseases and to provide immunity to certain diseases. Monocytes are involved in phagocytosis of pathogens and can develop into macrophages in tissues. Lymphocytes can mount immune responses by direct cell attack or by producing antibodies. Platelets are instrumental in blood clotting and can seal small tears in blood vessels; (Bondurant, M.C. and Koury, M.J. Origin and Development of Blood Cells, In: Lee, G.R., Foerster, J., Lukens, J., Paraskevas, F., Greer, J.P., and Rogers, G.M. (eds). Wintrobe's Clinical Hematology, 3rd Edition.

Early stage hematopoiesis involves the infrequent division of early stem cells to generate more stem cells, i.e. self-renewal. In an intermediate stage, the stem cells differentiate into progenitor cells that are irreversibly committed to produce only one or a few lineages of blood cells. In late stage hematopoiesis, the progenitor cells progressively proliferate and develop into terminally-differentiated and mature blood cells of a single lineage.

In humans, hematopoiesis appears to start in blood islands found in the fetal yolk sac during the first trimester of pregnancy. At about six weeks of gestation, hematopoiesis occurs predominantly in the fetal liver. In the beginning of the midtrimester, the bone marrow becomes the main site for hematopoiesis.

Hematopoietic stem cells are operationally defined as cells capable of self-renewal, and of differentiating into all types of mature blood cells. As a result, hematopoietic stem cells are capable of providing long-term hematopoietic reconstitution of ablated animals, including the repopulation of all myeloid and lymphoid cell lines.

Hematopoietic stem cells are morphologically small to medium mononuclear

(lymphocyte-like) cells. They have a large nuclear/cytoplasmic ratio, with prominent nucleoli and non-basophilic and agranular cytoplasm.

As stem cells reach the intermediate stage of hematopoiesis, they have less capacity for self-renewal and greater capacity for differentiation. Eventually, they become committed to develop into mature blood cells of a single lineage.

The late stage of hematopoiesis involves the formation of mature blood cells. The mature blood cells are not mitotic, and do not self-renew or differentiate. Each particular type of mature blood cell is ultimately committed, terminally differentiated, and limited to a single lineage.

Stromal Cells

In order to function properly, hematopoietic stem cells must be in intimate contact with stromal cells (Dexter, T.M., L.H., Spooncer, E., Heyworth, C.M., Daniel, C.P., Schiro, R., Chang, J., and Allen, T.D. 1990. "Stromal Cells in Hematopoiesis" in: Bock, G., Marsh, J. (eds), Symposium on Molecular Control of Haemopoiesis held at Ciba Foundation, London, John Wiley & Sons Ltd., Chichester, p 76-95). Stromal cells are produced in various organs where stem cells exist. Direct cell contact with stromal cells inhibits stem cells from differentiating and promotes self-renewal (Schofield, R. and Dexter, T.M. 1985. "Studies on the Self-Renewal Ability of CFU-S Which Have Been Serially Transferred in Long Term Culture or In Vivo," Leukemia Res. 9:305-313; Dexter, T.M., Spooncer, E., Simons, P., and Allen, T.D. 1984. "long-term Marrow Culture: An Overview of Techniques and Experience," in: Wright, D.G., Greenberger, J.S. (eds), Long-Term Bone Marrow Culture, Alan R. Liss, Inc., New York, p 57-96).

Seven-Transmenbrane G-Protein Coupled Receptors

A variety of extracellular stimuli transmit signals through seven-transmembrane G-protein coupled receptors (7TM-GPCRs). Over 1000 members of this family of receptors have been identified and some have served as targets for developing therapeutic agents that block or enhance their function. Members of this receptor family are characterized by an extracellular N-terminus, seven membrane-spanning domain and a cytoplasmic C-terminus.

The primary function of 7TM-GPCRs is to identify a specific signaling molecule or ligand from a large array of chemically diverse extracellular substances. Once identified, these cell-surfaced receptors can activate an effector-signaling cascade that triggers an intracellular

response and eventually a biological effect.

7TM-GPCRs undergo a conformational change upon binding with a ligand. The conformational change allows the receptors to associate with, and activate, heterotrimeric G-proteins. The G-proteins bind guanine and act to modulate intracellular signal pathways by interacting with a variety of effector molecules. The signals transduced lead to the regulation of important biological processes such as cell growth and differentiation. Disregulated cellular signaling through 7TM-GPCRs can contribute to human disease.

Objectives

A better understanding of stem cells, stromal cells, and their interactions would lead to a better understanding of aberrant regulation and diseases affecting the blood system, which, in turn, would lead to cures of such aberrant regulation and diseases. In addition, it is desirable to gain further insight into the molecular mechanisms underlying the different stages of hematopoietic development. It is also desirable to identify novel human nucleic acid molecules that may be involved in the molecular biology of hematopoiesis or play a role in hematopoietic differentiation or lineage commitment of cells that express such nucleic acid molecules. Additional information on the molecular biology of hematopoiesis is desired in order to improve the transplant therapeutic strategies for the treatment of acquired and genetic disorders of the hematopoietic systems.

7TM-GCPRs are involved in cellular signaling. Increased knowledge of 7TM-GPCRs would lead to a better understanding of cellular signaling. Cellular signaling leads to the regulation of important biological processes such as growth and differentiation. Additional information on the regulation of biological processes such as growth and differentiation would be very beneficial in developing treatments for various human diseases.

SUMMARY OF THE INVENTION

These and other objects as will be apparent to those having ordinary skill in the art have been met by providing an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 387. The invention further includes vectors and host cells comprising such nucleic acid molecules.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The nucleic acid molecules of the invention are selectively expressed in hematopoietic stem cells or stromal cells. For the purposes of this specification, hematopoietic stem cells are cells that are capable of differentiating at least into erythrocytes, neutrophils, basophils, eosinophils, monocytes, lymphocytes, and platelets. Mature hematopoietic cells are progenitor cells that are committed to a particular type of hematopoietic cell or to fully differentiated hematopoietic cells.

Nucleic acid molecules include both DNA and RNA. A nucleic acid molecule is isolated if it is removed from a cell, such as, for example, the cell in which the nucleic acid molecule is naturally found, and at least some of the other nucleic acid molecules that are found in the cell.

Preferably, the nucleic acid molecule is purified. A nucleic acid molecule of the invention is considered purified if it is substantially free from other nucleic acid molecules that are found naturally in the same cell as the nucleic acid molecule of the invention.

A nucleic acid molecule of the invention is considered substantially free from other nucleic acid molecules if the nucleic acid molecule of the invention constitutes at least about 50%, preferably at least about 60%, more preferably at least about 70%, most preferably at least about 80%, and optimally at least about 90%, or even higher, such as about 95%, about 98%, or even about 99% of the total weight of a mixture of nucleic acid molecules.

The nucleic acid molecule may consist of the nucleotides shown in a particular sequence, i.e., not contain any nucleotides other than those shown in SEQ ID NO: 1-387. Alternatively, the nucleic acid molecule may comprise the nucleotides shown in a particular sequence, i.e., include the nucleotides shown in SEQ ID NO: 1-387 as well as nucleotides other than those shown in the SEQ ID NO: 1-387 at either, or both, the 5' or the 3' end of the nucleic acid molecule. Such other nucleotides may include, for example, regulatory elements, such as transcription factors, translation factors, polyadenylation signals, transcription termination sequences, translation termination sequences, transport signals, promoters, transcription enhancers, activation sequences, and the like.

For example, the nucleic acid molecule may be incorporated into a vector. In this specification, a vector is a nucleic acid molecule that comprises an isolated nucleic acid molecule

according to the invention, and that is useful for transfecting cells. The vector may be linear or circular, i.e. a plasmid. Some examples of vectors include cloning vectors and expression vectors.

The invention also includes the nucleic acid molecule shown in SEQ ID NO: 386. This nucleic acid molecule is a full reading frame that encodes a 7TM-GPCR when expressed. (SEQ NO ID: 389)

In addition, the invention includes the nucleic acid molecule shown in SEQ ID NO: 387. This nucleic acid molecule represents the full reading frame of SEQ ID NO: 173. The reading frame for SEQ ID NO: 387 encodes a 7TM-GPCR when expressed. (SEQ ID NO: 388)

The invention also includes antibodies generated against the proteins represented by SEQ. ID NO: 388 or SEQ. ID NO: 389. The antibodies may be polyclonal or monoclonal.

Northern analysis of the nucleic acid molecule of SEQ ID NO: 386 reveals wide expression in the tissue of the brain, heart, lung and kidneys, as well as murine thymus and whole bone marrow.

Northern analysis reveals the nucleic acid molecule of SEQ ID NO: 387 to be expressed in murine bone marrow, thymus and a monoblastic leukemia cell line. The nucleic acid of SEQ ID NO. 387 appears to be restricted to hematopoietic tissues. Further studies reveal that the nucleic acid of SEQ ID NO. 387 is expressed in myeloblastic leukemia (M1) cells.

The invention further includes host cells comprising a nucleic acid molecule according to the invention. The nucleic acid molecule may be in a vector that has been transfected into the host cell, such as a cloning vector or an expression vector. Alternatively, the nucleic acid molecule may be incorporated into the genome of the host cell. In either event, the host cell preferably replicates or expresses the nucleic acid molecule, and more preferably both replicates and expresses the nucleic acid molecule.

The host cells may be used to express the polypeptides and proteins encoded by the nucleic acid molecules of the invention. Further, antibodies may be raised against epitopes of the expression products.

Equivalents

The nucleic acid molecules of the invention include homologs of the nucleic acid sequences provided in this application. In the present specification, the sequence of a first

nucleotide sequence is considered homologous to that of a second nucleotide sequence if the first sequence is at least about 60% identical, preferably at least about 70% identical, and more preferably at least about 75% identical to the second nucleotide sequence. In the case of nucleotide sequences having high homology, the first sequence is at least about 80%, preferably at least about 85%, more preferably at least about 95%, and optimally at least about 98% or 99% identical to the second nucleotide sequence.

In order to compare a first nucleic acid sequence to a second nucleic acid sequence for the purpose of determining homology, the sequences are aligned so as to maximize the number of identical nucleotides. The sequences of homologous nucleic acid molecules can usually be aligned by visual inspection. If visual inspection is insufficient, the nucleic acid molecules may be aligned in accordance with the methods described by George, D.G. et al., in *Macromolecular Sequencing and Synthesis*, *Selected Methods and Applications*, pages 127-149, Alan R. Liss, Inc. (1988), such as formula 4 at page 137 using a match score of 1, a mismatch score of 0, and a gap penalty of -1.

An alternative test for homology of two nucleic acid sequences is whether they hybridize under normal hybridization conditions, preferably under stringent hybridization conditions.

The term "stringent conditions," as used herein, is equivalent to "high stringent conditions" and "high stringency." High stringent conditions are defined in a number of ways. In one definition, stringent conditions are selected to be about 25°C lower than the thermal melting point (T_m) for DNA or RNA hybrids longer than 70 bases, and 5°C lower than the T_m for shorter oligonucleotides (11-70 bases long). The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched sequence. Typical stringent conditions are those in which the salt concentration is about 0.02 M at pH 7.0 and the temperature is calculated as described below.

The following equations are used to calculate the T_m of the following hybrids at pH 7.0: For DNA hybrids of more than 70 nucleotides: $T_m = 81.5^{\circ}\text{C} + 16.6 \log [\text{M}^{+}] + 41(\%\text{G} + \text{C}) - 0.63(\% \text{ formamide}) - (600/L)$. For DNA:RNA hybrids of more than 70 nucleotides: $T_m = 79.8^{\circ}\text{C} + 18.5 \log[\text{M}^{+}] + 58.4(\%\text{G} + \text{C}) + 11.8(\%\text{G} + \text{C})^2 - 0.5(\% \text{ formamide}) - 820/L$. For DNA or RNA hybrids of 14-70 bases: $T_m = 81.5^{\circ}\text{C} + 16.6 \log [\text{M}^{+}] + 41(\%\text{G} + \text{C}) - 600/L$. For DNA or RNA hybrids of 11-27 bases (based on 1 M Na⁺ and in the complete absence of organic solvents): $T_m = 4(\%\text{G} + \text{C}) + 2(\%\text{A} + \text{T/U})$.

Where

 $T_{\rm m}$ = thermal melting temperature;

%G+C = percentage of total guanine and cytosine bases in the DNA, usually

30% -75% (50% is ideal), and expressed as a mole fraction;

[M⁺] = monovalent cation concentration, usually sodium, expressed in molarity in the range of 0.01 M to 0.4 M; and

L =length of the hybrid in base pairs;

%A+T = mole fraction of total adenine and thymine bases in the DNA.

%A+T/U = mole fraction of total adenine and thymine or uracil bases in the DNA or RNA.

Some examples of "stringent conditions" useful in the present invention include overnight incubation at a hybrid temperature determined as described above in a solution comprising: 20% formamide, 5 x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA. Alternatively, the stringent conditions are characterized by a hybridization buffer comprising 30% formamide in 5 x SSPE (0.75 M NaCl, 0.05 M NaPO₄, pH 7.7, 5 mM EDTA) buffer at a temperature determined as described above and subsequent washing at the same temperature with 0.2 x SSPE. Preferably, stringent conditions involve the use of a hybridization buffer comprising 50% formamide in 5 x SSPE at a temperature determined as described above and washing at the same temperature with 0.2 x SSPE (0.03 M NaCl, 2 mM NaPO₄, pH 7.7, 0.2 mM EDTA).

SEQ ID NOS: 1-387 were derived from murine hematopoietic stem cells (SEQ ID NO: 1-248), or stromal cells (SEQ ID NO: 249-387). The nucleic acid molecules of the invention further include homologous sequences found in humans in accordance with the definitions of homology described above. The human sequences are derived from the same types of cells as the corresponding murine sequences, share homology, generally high homology, with the corresponding murine sequences, and are useful in the same ways as the corresponding murine sequences.

Human Equivalents

Positive human cDNA clones may be isolated and sequence analysis of the clones may be performed by methods known in the art. Sequence comparisons may be carried out by screening several databases, e.g., dbEST, GenBank, Swiss-Prot, and EMBL.

The human nucleic acid molecules screened by the methods described above may be from human cDNA or genomic libraries, preferably derived from hematopoietic cells or cell lineages. Particularly useful cells for this purpose include hematopoietic cells that are CD34+, CD38-, lin-, or any combination thereof. Such cells may be enriched for hematopoietic stem cells from bone marrow cells by standard methods well known in the art by which nucleated non-adherent cells are prepared to provide a rich source of hematopoietic stem cells.

Stromal cells may also be prepared from bone marrow cells by a similar process, this time selecting adherent cells. Larger numbers of stromal cells may also be prepared from umbilical cord blood. Another commonly used and readily available source of human cDNAs is the commercially available human liver cDNA libraries.

Utility

The nucleic acid molecules of the invention are useful in numerous ways. For example, since hematopoietic stem cells selectively express the nucleic acid molecules of the invention, assays that are capable of determining the presence of the nucleic acid molecules in a sample are capable of distinguishing such cells from most, if not all, other types of cells, such as mature hematopoietic cells and non-hematopoietic cells.

Thus, if a nucleic acid molecule of the invention, or its complement, is expressed in a cell, the cell is considered to have a high likelihood of being a hematopoietic stem cell. Methods for determining whether a nucleic acid molecule is expressed in a cell are known in the art. For example, high molecular weight DNA from a cell can be restricted with restriction enzymes and fractionated by agarose gel electrophoresis. The restricted fragments are denatured, transferred to a nitrocellulose filter, and immobilized (Southern transfer). A labeled nucleic acid molecule of the invention or its complement is prepared and used as a probe. The presence of the labeled probe hybridized to an immobilized nucleic acid molecule indicates the presence of the nucleic acid molecule in the sample.

Alternatively, the labeled probe is applied directly to fixed, denatured, and dehydrated

hematopoietic cells to localize the cellular transcripts in the cells and to identity the cell types that transcribe the mRNA of interest (in situ hybridization). Examples of these methods are described by Ausubel, F.M. et al. (eds), Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York (1999).

Nucleic acid molecules of the invention can be labeled by methods known in the art. The label may be a radioactive atom, an enzyme, or a chromophoric moiety.

Methods for labeling oligonucleotide probes have been described, for example, by Leary et al., Proc. Natl. Acad. Sci. USA, 80:4045 (1983); Renz and Kurz, Nucl. Acids Res. 12:3435 (1984); Richardson and Gumport, Nucl. Acids Res., 11:6167 (1983); Smith et al., Nucl. Acids Res. 13:2399 (1985); Meinkoth and Wahl, Anal. Biochem., 138:267 (1984); and Ausubel, F.M. et al. (Eds.) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York, 1999.

The label may be radioactive. Some examples of useful radioactive labels include ³²P, ¹²⁵I, ¹³¹I, ³⁵S, ¹⁴C, and ³H. Uses of radioactive labels have been described in U.K. 2,034,323, U.S. 4,358,535, and U.S. 4,302,204.

Alternatively, the label may be non-radioactive. Some examples of non-radioactive labels include enzymes, chromophores, atoms and molecules detectable by electron microscopy, and metals detectable by their magnetic properties.

Some useful enzymatic labels include enzymes that cause a detectable change in a substrate. These useful enzymes and their substrates include, for example, horseradish peroxidase (pyrogallol and o-phenylenediamine), beta-galactosidase (fluorescein beta-D-galactopyranoside), and alkaline phosphatase (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium). The use of enzymatic labels have been described in U.K. 2,019,404, EP 63,879, in Ausubel, F.M. et al. (Eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York (1999), and by Rotman, Proc. Natl. Acad. Sci. USA 47:1981-1991 (1961).

Useful chromophores include, for example, fluorescent, chemiluminescent, and bioluminescent molecules, as well as dyes. Some specific chromophores useful in the present invention include, for example, fluorescein, rhodamine, Texas red, phycoerythrin, umbelliferone, luminol.

The labels may be conjugated to the nucleotide probe by methods that are well known in the art. The labels may be directly attached through a functional group on the probe. The probe either contains or can be caused to contain such a functional group. Some examples of suitable

functional groups include, for example, amino, carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate.

The nucleic acid molecules of the invention are also useful in ways other than those described above. For example, the nucleic acid molecules are useful in identifying nucleic acid molecules that comprise one or more of the sequences in SEQ. ID NO 1 to SEQ. ID NO 387 and additional nucleotides at the 5' end, the 3' end, or both the 5' and a 3' ends. Such longer nucleic acid molecules express proteins that are involved in hematopoiesis. Such proteins are important to improve the understanding of hematopoiesis, and may be important in treating conditions characterized by abnormal hematopoiesis.

Methods to identify longer nucleic acid molecules that comprise one or more of SEQ. ID NO: 1 to SEQ. ID NO: 385 are known in the art. For example, a suitable method is the polymerase chain reaction (PCR) method described by Saiki et al. in Science 239:487 (1988); Mullis et al. in U.S. Patent No. 4,683,195; and Ausubel, F.M. et al. (eds), Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York (1999).

For extension of the sequence of the nucleic acid of the present invention, the RACE (rapid amplification of cDNA ends) method for 5'-end extension is particularly useful. See, for example, Frohman, "RACE" in PCR Protocols: A Guide to methods and Applications (Innes, M.A. ed.) Academic Press, San Diego, pp28-38 (1990). Briefly, primers oriented in the 5' and 3' directions are chosen to produce overlapping cDNAs when fully extended. The overlapping 5' and 3' end RACE products are ligated to produce longer nucleic acid molecules, such as full length cDNAs and genes. See, for example, Frohman, M.A. "RACE: Rapid Amplification of cDNA Ends" in PCR Protocols, A Guide to Methods and Applications. Innis, M.A. et al. (eds), Academic Press, Inc., New York (1990).

In the PCR methods described above, nucleic acid molecules of the invention are used as primers for PCR amplification. The oligonucleotide primers may be synthesized by methods known in the art. Suitable methods include those described by Caruthers in Science 230:281-285 (1985) and DNA Structure, Part A: Synthesis and Physical Analysis of DNA, Lilley, D.M.J. and Dahlberg, J.E. (eds), Methods Enzymol., 211, Academic Press, Inc., New York (1992). The amplified fragment may be cloned, sequenced and further amplified to obtain longer nucleic acid molecules that comprise one or more of the sequences in SEQ. ID NO: 1 to SEQ. ID NO: 385. It is convenient to amplify the clones in the lambda-gt10 or lambda-gt11 vectors using lambda-gt10

or lambda-gtll-specific oligomers as the amplimers (available from Clontech, Palo Alto, California).

The labeled murine probes can be used by various methods known in the art to screen human hematopoietic tissues or cells. Such screening can, for example, show where and how the human gene is being expressed and transcribed (northern blot analysis). See Sambrook, J. et al. (eds), Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989) and Ausubel, F.M. et al. (eds), Current Protocols in Molecular Biology, John Wiley & Sons, New York (1999).

The murine nucleic acid molecules of the invention may also be used as probes and used to isolate the corresponding human homolog by methods known in the art. For example, the murine nucleic acid molecules may be used as PCR primers to screen a human DNA (cDNA or genomic) library. The PCR methods described above, such as RAGE, are suitable for this purpose. See, for example, Zeng et al., Biochem. Biophys. Res. Comm., 236, 389-395 (1997), and Morita et al., Biochem. Biophys. Res. Comm., 248, 307-314 (1998). See also Buanne et al., Genomics 51, 233-242 (1998).

Alternatively, the nucleic acid molecules may be immobilized and used to capture human nucleic acid molecules having homology, preferably high homology, as described above from an appropriate human nucleic acid library. Methods for using nucleic acid molecules to immobilize and capture homologous nucleic acid molecules are known in the art. The conditions used in the capture procedure are highly stringent, as described above.

Another utility of the nucleic acid molecules of the invention is the mapping of DNA sequences specific to hematopoietic stem cells to chromosomes. The sequences may be mapped to a particular chromosome, or to a specific region of the chromosome by techniques known in the art. An example of a useful technique is fluorescent in situ hybridization (FISH) analysis. See, for example, Ausubel, F.M. et al. (eds), Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York (1999); Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, and U.S. Patent 5,447,841 of Gray et al.

The DNA of SEQ. ID NOS: 386 and 387 and the proteins corresponding to them in SEQ. ID NOS: 388 and 389 may be used to identify the types of cells in which they are expressed. These sequences can also be used to investigate cellular signaling between 7TM-GPCRs. Furthermore, the sequences may be helpful in the identification of new 7TM-GPCRs.

Additionally, the DNA of SEQ ID NOS. 386 and 387 encode for 7TM-GCPRs. 7TM-GPCRs are expressed on stem cells. Therefore, the DNA of SEQ ID NOS. 386 and 387 could serve as markers that can be used to sort stem cells. Sorted stem cells may be therapeutically useful, for example, in transplantations.

The 7TM-GCPRs corresponding to the DNA of SEQ ID NOS. 386 and 387 are expressed in myeloblastic leukemia (M1) cells, and play a role in leukemia. Hence, the development of inhibitors to the 7TM-GPCRs that correspond to the DNA of SEQ ID NOS. 386 and 387 provide a promising treatment to inhibit leukemia progression. This inhibition can occur via growth or differentiation inhibition.

In addition, the DNA of SEQ ID NOS. 386 and 387 and the proteins corresponding to them in SEQ. ID. NOS 388 and 389 may be used in the development of inhibitors of leukemic progression. The 7TM-GPCR corresponding to SEQ ID NOS. 386-389 is expressed in leukemia cells, particularly, myeloblastic leukemia (M1) cells. Therefore, development of inhibitors to the 7TM-GPCR will also inhibit leukemic progression. The inhibition of leukemic cells can occur via agonists or antagonists of the 7TM-GPCR that may induce differentiation as an anti-leukemia therapy.

Antisense Oligonucleotides

The present invention provides antisense or sense oligonucleotides capable of binding to a target mRNA or to the sequence in the double stranded DNA helix of SEQ ID NOS. 386 and 387. Antisense or sense oligomers according to the present invention, comprise a fragment of the coding region of the cDNA of SEQ ID NOS. 386 and 387. Such a fragment generally comprises at least 14 nucleotides, preferably about 14 to 30 nucleotides. The ability to create an antisense or sense oligonucleotide based upon a cDNA sequence for a given protein is known in the prior art and described in, for example, Stein and Cohen, Cancer Res. 48:2659, 1988 and van der Krol, et al., BioTechniques 6:958, 1988.

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of complexes that block translation (RNA) or transcription(DNA) by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of the proteins of SEQ ID NOS. 388 and 389 which correspond to the nucleic acids of

SEQ ID NOS. 386 and 387.

The antisense or sense oligonucleotides of the present invention further comprise oligonucleotides having modified sugar-phosphodiester backbones or other sugar linkages, which resist enzymatic degradation but retain sequence specificity to be able to target nucleotide sequences.

Antisense or sense oligonucleotides of the present invention may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including for example, CaPO₄-medited DNA transfection, electroporation, or by using gene transfer vectors. Antisense or sense oligonucleotides of the present invention also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, for example, cell surface receptors, growth factors, other cytokines or ligands that bind cell surface receptors.

Isolated and purified DNA of SEQ ID NOS. 386 and 387, or fragments thereof, may also be useful themselves as therapeutic agents in regulating the corresponding proteins of SEQ ID NOS. 388 and 389, which belong to the family of 7TM-GCPRs.

Antibodies

The present invention provides antibodies and/or functional equivalents of antibodies raised against the proteins represented by the amino aid sequences of SEQ. ID NO: 388 and SEQ. ID NO: 389. An antibody is defined as a protein that binds specifically to an epitope. The antibody may be polyclonal or monoclonal. The invention further includes isolating neutralizing antibodies that specifically recognize and bind to the proteins and functional analogs of the invention.

For this application, the functional equivalent of an antibody is preferably a chimerized or humanized antibody. A chimerized antibody comprises the variable region of a non-human antibody and the constant region of a human antibody. A humanized antibody comprises the hypervariable region (CDRs) of a non-human antibody. The variable region other than the hypervariable region, e.g. the framework variable region, and the constant region of a humanized antibody are those of a human antibody.

Suitable variable and hypervariable regions of non-human antibodies may be derived from antibodies produced by any non-human mammal in which monoclonal antibodies are made.

Suitable examples of mammals other than humans include, for example, rabbits, rats, mice, horses, goats, or primates. Preferably, the antibodies are human antibodies. The antibodies may be produced in a transgenic mouse. An example of such a mouse is the so-called XenoMouseTM (Abgenix, Freemont, CA) described by Green, LL., "Antibody Engineering Via Genetic Engineering of the Mouse: XenoMouse Stains are a Vehicle for the Facile Generation of Therapeutic Human Monoclonal Antibodies," *J. Immunol. Methods*," 10;231(1-2):11-23(1999).

Functional equivalents of antibodies further include fragments that have binding characteristics that are the same as, or are comparable to, those of the whole antibody. Suitable fragments of the antibody include any fragment that comprises a sufficient portion of the hypervariable (i.e. complementary determining) region to bind specifically, and with sufficient affinity, to 7TM-GPCR.

The preferred fragments are single chain antibodies. Single chain antibodies are polypeptides that comprise at least the variable region of the heavy chain of the antibody and the variable region of the light chain, with or without an interconnecting linker.

The antibodies and functional equivalents may be members of any class of immunoglobins, such as: IgG, IgM, IgA, IgD or IgE, and the subclass thereof. The functional equivalents may also be equivalents of combinations of any of the above classes and subclasses.

Methods for making monoclonal antibodies include, for example, the immunological method described by Kohler and Milstein in *Nature* 256:495-497 (1975) and by Camplbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon, et al., Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985). The recombinant DNA method described by Huse, et al. in *Science* 246:1275-1281 (1989) is also suitable.

Briefly, in order to produce monoclonal antibodies, a host mammal is inoculated with a receptor or a fragment of a receptor, as described above, and t hen, optionally, boosted. In order to be useful, the receptor fragment must contain sufficient amino acid residues to define the epitope of the molecule being detected. If the fragment is too short to be immunogenic, it may be conjugated to a carrier molecule. Some suitable carrier molecules include keyhold limpet hemocyanin and bovine serum albumin. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule.

Spleens are collected from the inoculated mammals a few days after the final boost. Cell suspensions from the spleen are fused with a tumor cell. The resulting hybridoma cells that express the antibodies are isolated, grown and maintained in culture.

Suitable monoclonal antibodies as well as growth factor receptor tyrosine kinases for making them are also available from commercial sources, for example, from Upstate Biotechnology, Santa Cruz Biotechnology of Santa Cruz, California, Transduction Laboratories of Lexington, Kentucky, R&D Systems Inc of Minneapolis, Minnesota, and Dako Corporation of Carpinteria, California.

Methods for making chimeric and humanized antibodies are also known in the art. For example, methods for making chimeric antibodies include those described in U.S. patents by Boss (Celltech) and by Cabilly (Genentech). See U.S. Patent Nos. 4,816,397 and 4,816,567, respectively. Methods for making-humanized antibodies are described, for example, in Winter, U.S. Patent No. 5,225,539.

Antibodies or antibody fragments can also be isolated from antibody phage libraries generated using techniques, for example, described in McCafferty et al., *Nature*, 348: 552-554 (1990), using the antigen of interest to select for a suitable antibody or antibody fragment.

Clackson et al., *Nature*, 352: 624-628 (1991) and Marks et al., J. Mol. Biol., 222: 581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries.

Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Mark et al., Bio/Technol. 10: 779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids Res.*, 21: 2265-2266 (1993)). These techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of "monoclonal" antibodies (especially human antibodies).

The preferred method for the humanization of antibodies is called CDR-grafting. In CDR-grafting, the regions of the mouse antibody that are directly involved in binding to antigen, the complementarity determining region or CDRs, are grafted into human variable regions to create "reshaped human" variable regions. These fully humanized variable regions are then joined to human constant regions to create complete "fully humanized" antibodies.

In order to create fully humanized antibodies that bind well to an antigen, it is advantageous to design the reshaped human variable regions carefully. The human variable

regions into which the CDRs will be grafted should be carefully selected, and it is usually necessary to make a few amino acid changes at critical positions within the framework regions (FRs) of the human variable regions.

For example, the reshaped human variable regions may include up to ten amino acid changes in the FRs of the selected human light chain variable region, and as many as twelve amino acid changes in the FRs of the selected human heavy chain variable region. The DNA sequences coding for these reshaped human heavy and light chain variable region genes are joined to DNA sequences coding for the human heavy and light chain constant region genes, preferably $\gamma 1$ and κ , respectively. The reshaped humanized antibody is then expressed in mammalian cells and its affinity for its target compared with that of the corresponding murine antibody and chimeric antibody.

Methods for selecting the residues of the humanized antibody to be substituted and for making the substitutions are well known in the art. See, for example, Co et al., Nature 351:501-502 (1992); Queen et al., Proc. Natl. Acad. Sci. 86: 10029-1003 (1989) and Rodrigues et al., Int. J. Cancer, Supplement 7: 45-50 (1992). A method for humanizing and reshaping the 225 anti-EGFR monoclonal antibody described by Goldstein et al. in PCT application WO 96/40210. This method can be adapted to humanizing and reshaping antibodies against other growth factor receptor tyrosine kinases.

Methods for making single chain antibodies are also known in the art. Such methods include screening phage libraries transfected with immunoglobulin genes described in U.S. Patent 5,565,332; U.S. Patent 5,5837,242; U.S. Patent 5,855,885; U.S. Patent 5,885,793; and U.S. Patent 5,969,108. Another method includes the use of a computer-based system for designing linker peptides for converting two separate polypeptide chains into a single chain antibody described in U.S. Patent 4,946,778; U.S. Patent 5,260,203; U.S. Patent 5,455,030; and U.S. Patent 5,518,889.

Other methods for producing the functional equivalents of antibodies described above are disclosed by Wels et al. in European patent application EP 502 812 and *Int. J. Cancer* 60:137-144 (1995); PCT Application WO 93/21319; European Patent Application 239 400, PCT Application WO 89/09622; European Patent Application 338 745; U.S. Patent 5,658,570; U.S. Patent 5,693,780; and European Patent Application EP 332 424.

The antibodies that bind specifically to the proteins comprising the amino acid sequences

of SEQ ID NOS: 388 and 389 can be used to detect the presence of said proteins. Assays for detecting the presence of proteins with antibodies can be performed using known formats such as standard blot and ELISA formats.

The antibodies that bind specifically to the protein comprising the amino acid sequence of SEQ ID NO. 388 can be used to identify murine bone marrow and thymus cells as well as cells of the monoblastic leukemia cell line in which it is expressed. The antibodies corresponding to the protein comprising the amino acid sequence of SEQ ID NO. 389 can be used to identify brain, heart, lung and kidney cells.

The antibodies of the invention may be used therapeutically as cytotoxic agents against, for example, leukemia cells. Antibodies with proper biological properties are useful directly as therapeutic agents. See, for example, U.S. Patent No. 5,134,075. Alternatively, the antibodies can be bound to a toxin to form an immunotoxin or to a radioactive material or drug to form a radiopharmaceutical or pharmaceutical. Methods for producing immunotoxins and radiopharmaceuticals of antibodies are well-known. See, for example, Cancer Treatment Reports (9184) 68: 317-328.

EXAMPLES

Hematopoietic Stem Cell Isolation /Sorting and Transplantation:

AA4+Sca1+c+kit+Lin-/lo (hematopoietic stem cell-enriched) and AA4- (hematopoietic stem cell depleted) cells were isolated by fluorescence activated cell sorting from the fetal liver of C57BL/6j-Ly-5.2 mice on day 14 of gestation as follows: quantitative enrichment of stem cell fractions was performed according to described protocols. Fetal livers were dissected from day 14 embryos in a sterile environment. Single cell suspensions were subjected to immunopanning with AA4.1 antibody. The AA4.1 positive fraction was then stained with fluorescein isothiocyanate (FITC)-labeled rat anti-CD3, CD4, CD5, CD8, CD45R, Gr-1 and TER-119 antibodies (called the "lin" set of antibodies). Cells were also stained with phycoerythrin (PE)-conjugated anti-Sca-1 (Ly-6A/E) and allophycocyanin (APC0-conjugated anti-c-kit. All antibodies were obtained from Pharmingen (San Deigo, CA). Stained cells were separated on a dual laser EPICS 753 cell sorter (Beckman Coulter, San Diego, CA) by selection of high PE and APC as well as low FITC fluorescence. Cells used for cDNA library production were tested for

their stem cell potential in competitive repopulation assays that test the ability of stem cells to reconstitute the radioblated hematopoietic system of recipient mice.

This transplantation assay was performed essentially by methods known in the art. For the fetal liver, specified numbers of cells (25, 50, 100 or 250) were mixed with 400,000 C57B1/6-Ly-5.1 whole bone marrow cells and injected into the retro-orbital sinus of each irradiated (10 Gy) mouse. The level of engraftment was measured at different timepoints as the percent C57b1/6J-Ly-5.2 (donor) cells in the peripheral blood of transplanted Ly-5.1 animals. Animals demonstrating engraftment at least two standard deviations higher than the mean of negative controls were considered positive.

CDNA Library Construction and Subtractive Hybridization

Sorted cells were placed directly into Trizol (BRL-Gibco) reagent and treated with RNAse-free DNAse I according to the manufacturers protocols. The polyA-plus fraction was purified on oligo-dT cellulose (New England Biolabs). Prior to first strand cDNA synthesis, the mRNA was denatured using methyl-mercury hydroxide. The first and second strand cDNA was synthesized using the reverse transcriptase SuperScript II, DNA polymeraseI, RNAseH and *E.coli* ligase. The first-strand primer was oligo-dT with a 5' Notl site. Double-stranded cDNA was blunt-ended with T4-DNA polymerase and ligated to a Sall adapter with T4-DNA ligase. The ligated cDNA was digested with Notl and size fractionated on columns. Size fractions 1 kilobase pair (kbp.) and greater in length were pooled and ligated in to the Sall-Notl sites of either pSport-1 (stem cell library) or pSport-2 (mature cell library). The ligations were electroporated into *E.coli* strain DH12S, titered and amplified on agar plates. Except where indicated, all of the reagents and protocols for the construction of the cDNA were from BRL-Gibco.

The basic strategy used to perform the subtractions has been described (Rubenstein et al. 1990, Li et al. 1994). Briefly, the stem cell library was converted into single stranded molecules by *in vivo* infection with helper phage according to BRL-Gibco protocols. Cesium gradient banded DNA from the mature cell library was linearized with Sall and used as template for the synthesis of biotinylated RNA using T7-polymerase. The use of the two different cloning plasmids insures that the RNA is complementary to the single-stranded cDNA inserts in the stem cell library. Hybridization conditions and the post-hybridization processing of the reactions were essentially as described in the BRL-Gibco protocols. Two successive rounds of hybridization

were performed. The resultant subtracted single-stranded DNA populations were repaired to a double-stranded from and amplified in *E.coli* DH12S. The DNA populations representing the subtracted libraries were purified on Cesium gradients. In order to eliminate the plasmids which did not contain inserts (these are enriched following subtraction), the library DNAs were linearized with Notl and subjected to four successive agarose gel electrophoresis fractionations. After each electrophoresis gel the DNA smear corresponding in size to vector plus 1 kbp and greater was excised and eluted using the sodium iodide/glass bead procedure. After four such fractionations essentially all empty plasmids were eliminated.

The resultant DNA population was introduced into *E.coli* DH12S and individual clones were robotically picked into 384-well plates. A separate aliquot of the library was amplified as a population and used to prepare DNA. The subtraction efficiencies were verified by monitoring the clone number reduction as a function of hybridization with biotinylated RNA in comparison with mock hybridizations lacking RNA. Generally, this was on the order of 100-fold. A more direct measure of subtraction efficiency was obtained by hybridizing pre and post-subtraction cDNA Southern blots with probes such as beta-actin, CD34 and flk-2. The DNA populations on the blots represent approximately equal numbers of individual clones. The cDNAs corresponding to the CD34 and flk-2 molecules (both previously shown to be expressed preferentially in the stem cell-enriched populations are enriched or at least retained in the subtracted libraries. Special care was taken to carefully monitor the minimal number of PCR cycles necessary to produce the required amount of amplified product. This was done by performing pilot reactions using 2 cycle increments.

The amplified material representing different numbers of PCR cycles were analyzed in triplicate Southern blots that were hybridized with probes representative of different mRNA size classes. In various experiments these included GAPDH, beta-actin, flk-2, CD34 and ckit. Cycle numbers where discrete full-length cDNA products were observed with little or no detectable lower molecular weight material were determined and, in general, the preparative amplifications employed 1 fewer cycles. The amplified cDNA material was used to generate libraries as described above. Alternatively, the cDNA was used for PCR-Select subtractions. These methods were performed by methods well known in the art.

DNA Sequencing

Initial sequences were obtained by chain termination using the Sequenase Version 2.0 kit (U.S. Biochemicals). The majority of randomly selected sequences were generated by single-pass automated sequencers by Commonwealth Biotechnologies, Inc. (Richmond, VA) or by Incyte Pharmaceuticals Inc. (Palo Alto, CA).

Biological Sequence Analysis

DNA sequences and conceptual translations were compared with known nucleotide and protein sequences using the BLAST algorithm (blastn for nucleotide and blastx for protein databases). Six publicly-accessible databases were searched: SwissProt, Genbank nr protein, Genbank nr nucleotide, dbEST expressed sequence tags, and the murine and human DOTS databases of EST contigs. Sequences were also compared with those in SCBD itself as a measure of internal redundancy. Potential open reading frames (ORFs) were located using ORF finder (NCBI, Bethesda, MD). Protein motif searches were performed using five different motif identification programs: Prosite (PBIL, France), Pfam (Washington University, St. Louis), ProDom (INRA, France), SMART (EMBL, Heidelberg, Germany) and eMatrix (Stanford University). Transmembrane helices were detached using the TMPred server, and potential signal peptides were detected with SignalP. Subcellular localizations were predicted in some cases using PSORT II.

CLAIMS

1. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:385.

- 2. An isolated nucleic acid molecule according to claim 1 wherein the nucleic acid molecule is a DNA molecule.
- 3. An isolated nucleic acid molecule according to claim 1 wherein the nucleic acid molecule is an RNA molecule.
- 4. An isolated nucleic acid molecule according to claim 1 wherein the nucleic acid molecule is purified.
 - 5. A vector comprising a nucleic acid molecule according to claim 1.
 - 6. A vector according to claim 2 wherein the vector is a cloning vector.
 - 7. A vector according to claim 2 wherein the vector is an expression vector.
 - 8. A host cell comprising a nucleic acid molecule according to claim 1.
 - 9. A host cell according to claim 8 wherein the nucleic acid molecule is in a vector.
 - 10. A host cell according to claim 8 wherein the vector is a cloning vector.
 - 11. A host cell according to claim 8 wherein the vector is an expression vector.
- 12. A host cell according to claim 8 wherein the nucleic acid molecule is incorporated into the genome thereof.

13. A host cell according to claim 8 wherein the host cell expresses the nucleic acid molecule.

- 14. An isolated nucleic acid molecule comprising SEQ. ID NO: 386.
- 15. An isolated nucleic acid molecule comprising SEQ. ID NO: 387.
- 16. An isolated protein comprising the amino acid sequence of SEQ ID NO. 388.
- 17. An isolated protein comprising the amino acid sequence of SEQ ID NO. 389.
- 18. An antibody that binds specifically to the protein according to claim 16.
- 19. An antibody that binds specifically to the protein according to claim 17.

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<212> DNA

<213> Mouse

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anagningnen etanangggg ntgathengn anacetanag eageetning theetninga 240
conntntatt nanaactntg atnatottca ntntngngng toganntttg cnannatago 300
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525

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89

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gaggcaagac acaactttgg gatcgtggag atagacggga tgctctacat ccttggaggg 180
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105

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  cccctgggt ggcatggtgc tcactgccag cttccctgcc cgaaggacca gtttngtgaa 360
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212> DNA

<213> Mouse

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gttgttctga ttttgatgct gggaagttta tttgcatttc atcatggnct gtatttnnan 360
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568

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639

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caaaacctgg acctggctca actggttage ctgtggcttc agtacctttt tgtttttctc 360
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ggaagtactt geteegeetg gatgteegea aggageaegt aaagttetgg gggeecaage 420
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tgctggncct ggtggggaaa cccccggggt gccctgcctc tgcttcgcct tggncaacca 480

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aqtcattanc actcingtig gggccgttga gccanntinn ngcaagnnet tintaggete 360
ttntggttca nnttcaagtg gagattncga atccattaag tgtatggggn actgtcccca 420
ganaaaccca ctgtggccag atctcactct acaggnannt tttggagatt tcccgagctg 480
tnnaagntet gagteeneen tgacattaan gnnggtenne agtenaataa caaaagenee 540
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cacagaccct ggattgtcat ggtctatgga atgtgggcct gctggtgtgt gctgggggcg 240
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acquireactg trogctgcct gtactacacc agettcagec tggagetgtg ceggeagect 480
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acnnagcaac aaatttnaat cagaattccc ttcttcatta actggaaaag tagctcctga 180
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tgtgcggtgg ttgctttgtg gctgcctgtg ctgctgntgc acgttagggt gcagtatgtg 300
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ttqcctqaat tccagatget caatttetgt etttgtetee ttettgeete etettgeage 360
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<211> 540
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<400> 372
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<400> 374
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65

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75

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/04700

A. CLA	SSIFICATION OF SUBJECT MATTER									
IPC(7)	: C12N 15/11, 15/63, 15/70, 15/82; C07K 14/0	0								
US CL	: 536/23.1; 435/320.1, 455, 468; 530/300, 350	entional electification and IDC								
According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED										
		hu alassificacion symbols)	······································							
	cumentation searched (classification system followed 36/23.1; 435/320.1, 455, 468; 530/300, 350	by classification symbols)								
U.S 3	30/23.1, 433/320.1, 403, 406, 330/300, 550									
Documentati	on searched other than minimum documentation to the	e extent that such documents are include	d in the lields searched							
										
Electronic da	ta base consulted during the international search (nan	ne of data base and, where practicable, s	earch (erms used)							
STN, EAST	•									
C. DOC	UMENTS CONSIDERED TO BE RELEVANT									
Category .	Citation of document, with indication, where ap		Relevant to claim No.							
Y,P	PHILLIPS et al. The genetic program of hematopoid Vol. 288, pages 1635-1640, entire document.	etic stem cells. Science. 02 June 2000,	1-17							
	,	and the state of t								
Y	LELIAS et al. cDNA cloning of a human mRNA pro- cells and with homology to a GDP-dissociation inhib		1-17							
	proteins. Proc. Natl. Acad. Sci. USA. February 199	¥ 1	1							
	document.									
Y	MIRAGLIA et al. A novel five-transmembrane hem-	etopojetic stem cell entigen: Isolation	1-17							
•	characterization, and molecular cloning. Blood. 15 l	•								
	pages 5013-5021, entire document.									
Y	MOORE et al. Hematopoietic activity of a stromal of	ell transmembrane protein containing	1-17							
•	epidermal growth factor-like repeat motifs. Proc. No	· · · · · · · · · · · · · · · · · · ·								
	94, pages 4011-4016, entire document.									
		Į.								
Further	documents are listed in the continuation of Box C.	See patent family annex.								
-	pecial categories of cited documents:	"T" later document published after the inte								
"A" document	defining the general state of the art which is not considered to be	date and not in conflict with the applic principle or theory underlying the inve								
	tler relevance		•							
"E" entiter at	plication or pasent published on or after the international filing date	"X" document of particular relevance; the considered novel or cannot be considered.								
"L" document	which may throw doubts on priority claim(s) or which is cited to	when the document is taken alone								
	the publication date of another chation or other special reason (as	"Y" document of particular relevance; the considered to involve an inventive ste								
specified		combined with one or more other suc	documents, such combination							
"O" document	t referring to an oral disclosure, use, exhibition or other means	being obvious to a person skilled in th	e art							
	published prior to the international filing date but later than the	"&" document member of the same patent	family							
	itte claimed	Date of mailing of the insertional and	erch manort							
Date of the s	actual completion of the international search	Date of mailing of the international sea	nen report							
		27 JUN 2001								
	ailing address of the ISA/US	Authorized officed model								
	nealesismer of Patents and Trademarks PCT	Michael Woodward	7							
	shington, D.C. 20231	Telephone No. (703)-308-0196	U							
Lacamile M	o. (703)305-3230	1 - 1- PROPERTY (103)-200-0120								

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/04700

Box I Observations where certain claims were found unsearchable (Continuation f Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-17, SEQ ID NOs:386-389
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/04700

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING: This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-17, drawn to nucleic acid molecules, vector molecules and host cells containing said nucleic acids and polypeptides.

Group II, claims 18 and 19, drawn to antibodies.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, udner PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I encompasses nucleic acids, polypeptides expressed thereby, vectors and host cells containg same, respectively. Group II, however, is directed to an antibody, which antibody undergoes recognition and binding reactions wherein what is bound is different from what is bound by the compositions of Group I. For example, the polypeptides of Group I do not bind the polypeptides of Group I as the antibody of Group II does. Thus, in summary, each of Groups I-II is directed to different special technical features and thus supports this lack of unity.

Additionally, some claims are directed to more than one invention inciding more than one sequence, which are deemed to lack unity of invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one invention to be searched, the appropriate additional search fees must be paid. These inventions are as follows: For Group I, the claims include a series of polynucleotides and/or the polypeptides encoded thereby as represented by the sequences of SEQ ID Nos: 1-389. Each of these polynucleotide sequences encodes a separate polypeptide and thus represents a separate gene. Therefore, each of these genes defines its own special technical feature. For Group II, the antibodies are against two different proteins with sequences of SEQ ID NOs: 388 and 389, and each of the proteins, thus each of the antibodies, has its own special technical feature. In summary, for Group I, one invention is a gene represented by one polynucleotide sequence and one polypeptide sequence encoded thereby, or only the polynucleotide sequence where no polypeptide sequence encoded thereby is claimed, or the polypeptide sequence where no polypeptide is claimed, and for Group II, one invention is an antibody against a protein with the sequence of either SEQ ID NO: 388 or SEQ ID NO:389.

During a telephone conversation with IRVING FEIT, AND LAUREN EMR, applicants elect to pay for searching an invention: Group I encompassing claims 1-17 and SEQ ID NOs: 387 and 388 in addition to Group I encompassing claims 1-17 and SEQ ID NOs: 386 and 389. Accordingly, Group I encompassing claims 1-17 and SEQ ID NOs: 386-389 are searched